Kentucky
Agricultural Experiment Station
University of Kentucky

ACIDOSIS OF PREGNANT EWES: SO-CALLED PREGNANCY DISEASE OF SHEEP.

BULLETIN NO. 354
(RESEARCH BULLETIN)

Lexington, Ky.
November, 1934

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BULLETIN NO. 354

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Acidosis of Pregnant Ewes: So-Called Pregnancy Disease of Sheep.

W. W. DIMOCK, DANIEL J. HEALY and F. E. HULL

For the past ten or fifteen years sheep breeders thruout Kentucky, especially in the Bluegrass Region, have had severe losses of ewes in the last months of pregnancy, from the so-called pregnancy disease of sheep. A similar disease has been observed in different parts of the United States (23) and in European countries. The disease occurs during the months of January and February, a few weeks previous to lambing, and may continue thruout the lambing period. In this country, the disease is generally known as pregnancy disease of sheep, while in Europe it is known as acute diffuse parenchymatous hepatitis (1), and pregnancy toxemia (12).

Several observers have offered explanations of the cause of the disease. Heavy feeding and lack of exercise were considered factors by Faust (2) in North Dakota. Observers in Colorado (3) were of the opinion that a bipolar microbe caused the disease but other observers, including ourselves, failed to find evidence of infection. Murillo and Izcara (1) consider the disease an infectious inflammation of the bile ducts and gall bladder. More recently Roderick and Harshfield (4) were of the opinion that the disease is primarily a toxemia of pregnancy, while Sampson, Gonzaga and Hayden (5) maintain that the disease is a ketosis.

The following flock histories illustrate conditions frequently observed in the field.

C.S. Flock of 393 ewes in good condition. Fed good mixed hay, alfalfa and clover, and on bluegrass pasture. Four ewes

dead, two down with symptoms of acidosis. Lambs taken from the two sick ewes, and ewes recovered. Flock fed bran, oats and alfalfa hay. No more losses.

J.W.P. Flock of 200 ewes. Fed ear corn. Some bran and oats, clover hay and on bluegrass pasture. Five ewes dead. Taken off ear corn and fed bran, oats and clover hay.

C.Sp. Flock of 200 ewes. Fed alfalfa hay and rye pasture. Three ewes dead and five sick with symptoms of acidosis.

J.P.S. Flock of 200 ewes. Fed sheaf oats, some ensilage and on bluegrass pasture. Three ewes dead and five sick.

A.G. Flock of 45 ewes. Fed clover hay and corn. Five ewes dead and four sick. Feed changed to clover hay, oats and bran.

J.H.C. Flock of 800 ewes. Fed hay until lambing season, then bran, oats and clover hay. Lost 15 ewes with acidosis.

J.T.W. Flock of 66 ewes. On bluegrass pasture. Lost 4 ewes. Feed changed to corn, oats, bran, fodder and clover or alfalfa hay.

Dr.S. Flock of 50 ewes. Fed corn and corn stover. Two sick ewes on Friday afternoon. On the following morning one ewe was dead, the other had lambed one lamb, and was up and well.

J.D.A. Flock of 40 ewes. On bluegrass pasture with very little hay. Two ewes dead and four sick, all four died later.

A.M.B. Flock of 150 ewes. On bluegrass pasture. Lost 15 ewes with acidosis. Feed changed to mixed hay, corn, bran and oats.

W.N.O. Flock of 100 ewes. On bluegrass pasture. Lost 60 ewes with acidosis. During the following year, with a flock of 100 ewes under similar conditions, lost 13 ewes. During the third year, with a flock of 100 ewes fed a well-balanced ration of bran, oats, oil meal and alfalfa or clover hay, lost none with acidosis. Three old ewes died of other causes. During the five years thereafter, on well-balanced ration, lost no ewes with acidosis.

H.P.H. Flock of several hundred ewes maintained each year for several years, lost an undetermined number of ewes

each year. During the season 1932-1933, had a flock of 450 ewes, on bluegrass pasture and alfalfa hay. Lost 50 ewes. During the season 1933-34, had a flock of 450 ewes—250 old ewes held over and 200 Western ewes added. On bluegrass pasture, alfalfa hay and one ear of corn per ewe per day. Had no acidosis. Lost 4 or 5 ewes from other causes.

C.W. Flock of 39 ewes. It was first visited on Jan. 6, 1928. Six ewes had already died; two more were found dead, and four others were sick with rather typical symptoms of acidosis, with temperatures of 104, 103.2, 103.5 and 104. Two ewes had been sick for three days, one for two days and one for one day. Two autopsies were performed on ewes dead twenty-four, and four hours, respectively. These autopsies showed an abundance of fat internally altho subcutaneous fat was not pronounced. The flock, as a whole, was in good flesh. This flock was housed in a large barn and had free access to a large pasture. There was a pond in the field not far from the barn and a pump at the barn. In addition to the pasture the ewes were fed on corn stover and, rather irregularly, on timothy hay and corn. The visit was on a mild winter day with some snow on the ground in protected places. The previous week had been somewhat colder. Stored in the barn where the sheep were kept was an abundance of baled hay, both clover and timothy, altho practically none had been fed to the sheep. This flock is mentioned because of the high mortality, 12 out of 39, nearly 31 percent. The owner was advised to feed a grain ration composed of equal parts by weight of corn, bran and oats, and all the timothy and clover hay that the ewes would eat. Tho all the sick ewes died, no other cases developed. The owner stated that no cases of acidosis had occurred in the flock of breeding ewes since the winter of 1928. The method of management and care of the ewes had remained the same, except that a ration of grain and a varity of roughage, including good hay, had been fed during the fall and winter.

The popular opinion that sheep are valuable for cleaning a pasture of weeds has created the practice of turning sheep into poor pastures to forage for themselves. In Central Kentucky

the weather varies considerably during the lambing season. Some seasons are mild while others are severe. When the season is severe, fall and winter pasture will not properly nourish pregnant ewes and during the mild seasons bluegrass pasture is barely sufficient for proper nourishment.

Improper feeding of pregnant ewes approaching term brings about depletion of both food and mineral reserves of the body. In recent years the importance of mineral elements necessary for growth and development of all animals has been recognized. Woodman and Evans (17) state that malnutrition of sheep on mineral-deficient pastures may be attributed to the lack of inorganic materials necessary for the normal functioning of the body. It appears that of all the mineral elements found in the animal body, calcium and phosphorus, in proper proportions, are the ones that the pregnant ewe is most likely to lack, in sufficient quantity. A diet deficient in calcium causes disordered cellular metabolism which, in turn, causes cellular degeneration; such a diet, if continued, upsets endocrine function, causes a profound disturbance of the entire metabolism, and a true acidosis results.

The absence of cases of acidosis in the Kentucky Experiment Station flock of breeding ewes for seventeen years, is valuable evidence that, with ewes maintained in a state of good nutrition the year round, on a well-balanced ration, the disease can be prevented. On the other hand, the occurrence of acidosis in an experimental flock, fed for the purpose of causing acidosis, is valuable evidence that the disease is primarily nutritional in origin. Lack of exercise and twin pregnancies have been considered predisposing causes of acidosis of pregnant ewes. Lack of exercise does not appear as a vital factor in the development of the disease in Kentucky. Several cases of acidosis have occurred in ewes bearing one lamb. Many ewes have borne twins, triplets and even quadruplets without having acidosis.

SYMPTOMS

The first noticeable evidence of acidosis in ewes in the field is that the sick ewe lags behind the rest of the flock. Where the

flock is confined in a stable or small yard, the ewe is observed to stand off by herself, and neither eat nor drink. The sick ewe shows evidence of incoordination, nervousness and irritability, an inability to get over small obstructions, a tendency to walk in a circle or to stand with the head pressed against some object in the pen. The ewe's eyes appear dull and lusterless and visual disturbance is quite evident. At first the respirations are labored and accelerated, later, shallow. The temperature varies as in normal ewes and is of no diagnostic value. According to our observations ewes affected with this condition, especially in early stages, are more apt to be constipated than to suffer from diarrhea.

As the condition progresses the animal is found lying down more or less constantly, grinding the teeth, trembling, and gradually becoming indifferent to her surroundings. Still later the animal is unable to arise without assistance. At first the ewe may rest on the sternum, with the head turned toward the body; later she lies on her side with the head and neck flexed dorsally and the legs extened. Frothy discharge may be observed from both the mouth and nostrils. Finally the animal passes into almost complete coma and may have convulsions or spasms at irregular intervals or when disturbed. The odor of acetone may be observed coming from the lungs or from the blood when blood samples are taken for analyses. The duration of the disease may be several days, even after complete coma develops.

On examination lambs were always found alive in ewes killed in an advanced stage of the disease. The genital tract showed that the cervix was closed and sealed normally and there was no tendency of it to dilate and facilitate the delivery of the lamb. In a few cases where the ewe gave birth to an apparently healthy lamb or lambs the ewe immediately showed improvement and in a few hours was up and apparently normal. In one case dilatation of the cervix was successful and the lambs were delivered. The lambs were dead but the ewe immediately showed improvement and recovered without further incident. In numerous cases, however, it was exceedingly difficult to cause premature birth. In cases in which sick sheep delivered lambs

Fig. 1. Sick ewe resting on sternum.

Fig. 2. Sick ewe prostrated.

without assistance or in which forced delivery was used, it was assumed that pregnancy was for full term.

PATHOLOGY

Autopsies were held on 106 ewes dead from acidosis or that were killed in an advanced stage of the disease. Thirty-five additional cases were examined in the field. In all cases the liver and kidneys were the only organs in which pathological changes were obserevd with any degree of regularity.

The liver was found quite uniformly enlarged, soft, friable and mottled, with irregular-shaped areas of a yellowish or grayish color. On microscopical examination the livers showed varying degrees of degeneration. In some cases the degenerated areas were scattered thruout the tissue; in others the degeneration involved the entire tissue of the section, while in still others the changes were distinctly interlobular altho extending into the lobules. The cells in the outer portion of the lobule showed distinct cellular fatty degeneration, while deeper in the lobule the cellular changes were of a pronounced granular type. This indicates that the primary cellular change was a typical granular degeneration and that the fatty degeneration followed.

In practically every case the kidneys were enlarged and swollen to the extent that upon cutting the capsule the kidney tissue protruded. The kidney tissue, as a whole, was soft, sometimes to the point of being pulpy, very dark in color and gorged with blood. Microscopical examination of kidneys from different ewes revealed a variety of changes varying from a simple granular degeneration, most pronounced in the convoluted tubes, to complete disintegration of the cytoplasm of the cells lining the tubules. Evidence of fatty degeneration was only occasionally observed. Occasionally the broken-down cellular material appeared as a dense, structureless mass in the lumen of the tubules. In some cases hemorrhages were observed here and there in the sections. The glomeruli were not, as a rule, appreciably abnormal except that occasionally an individual glomerulus showed congestion and hemorrhage, and in a few

glomeruli a granular exudate was observed within the capsule but separated from the cellular structure of the glomerulus.

Bacteriologic examinations of ewes dead from the disease or killed in an advanced stage, and inoculations of sheep, guinea pigs and mice with material from such cases, gave negative results. Under these circumstances the possibility of observing characteristic changes in the urine was considered. As a search of the literature failed to yield an urinalysis for normal ewes, the urine of forty normal ewes was examined. Twenty-nine of these ewes were pregnant, eight barren, while with three pregnancy was doubtful. The normal urinalysis determined and published (6) was as follows: Color, yellow; condition, clear; no precipitate; specific gravity, 1031; reaction, alkaline; urea, 2.5 percent; ammonia, 0.01 percent; total nitrogen, 1.4 percent; no albumin, no sugar and no acetone.

On examining the urine in eleven cases of pregnancy disease, in three of which the blood, also, was examined, Dimock, Healy and Bullard (7) found the urine acid or markedly acid (one exception), with a precipitate, with ammonia increased from eight to fifteen times the normal quantity, and with acetone and albumin present. As these results suggested an acidosis, in three cases the urinalyses were checked by blood examinations. Cullen (8) has shown that the addition of an equal volume of .02*N* HCl to normal blood plasma, from which the CO_2 has been exhausted, causes a relatively small increase in acidity, because of the presence of buffers in the plasma; whereas in cases of acidosis, where the buffers of the blood are exhausted, the increase in acidity is marked. Using Cullen's method, the plasma of twelve normal ewes was examined and the increased acidity on the addition of an equal volume of .02*N* HCl, measured. The average reaction of normal plasma from which the CO_2 had been exhausted was pH 8.40; while the average reaction of the same plasma, after the addition of an equal volume of .02*N* HCl, was pH 6.67, showing an increase in acidity equal to pH 1.73. (It will be remembered that as the acidity increases the pH value decreases.)

Upon examining the blood of three cases of so-called preg-

nancy disease, the addition of an equal volume of .02*N* HCl to the plasma increased the acidity from pH 8.40 to pH 6.01 in case 5, and to pH 5.47 in cases 6 and 7. Dimock, Healy and Bullard (7) therefore stated: "In the so-called pregnancy disease of sheep the condition is clearly an acidosis. Therefore the authors name it acidosis of pregnant ewes." Later Roderick and Harshfield, (4) and Sampson, Gonzaga and Hayden (5) confirmed the finding that the disease is characterized by an acidosis.

We agree with Sellards (9) that an acidosis is an impoverishment of the tissues and fluids of the body in fixed bases or in substances which readily give rise to fixed bases. The alkaline reserves of the tissues and fluids of the body available for neutralization by organic and other acids derived from metabolism are the carbonates and phosphates of sodium, potassium, magnesium and calcium and, under certain conditions, ammonia. As Mathews (10) points out, many physiologists regard the appearance of an increased quantity of ammonia in the urine as characteristic of acidosis. Of these bases calcium promised the greater possibility of a recognizable variation because of the smaller quantity present in the blood and the greater demand on the maternal calcium during the last few weeks of pregnancy. The present writers, after estimating the blood calcium and potassium in thirteen cases of this disease stated: (11) "In these cases of acidosis of pregnant ewes the blood calcium content is distinctly diminished and the potassium content greatly increased. At present it is not known that this increase of potassium would be injurious to the ewe. The deficiency in calcium content, however, indicates that pregnant ewes coming down with this disease were not properly nourished in regard to calcium."

During the past seven lambing seasons sixty-two cases of acidosis of pregnant ewes have been observed. In forty-six of these cases, or 74 percent, there was subnormal blood calcium, while in sixteen, or 25 percent, there was normal or slightly increased blood calcium, and of these sixteen cases fifteen showed increased blood phosphorus so that, with two exceptions,

the ratio Ca/P was always less than normal. In the case where this ratio was greater than normal the ewe recovered, and this was the only case which did recover. Roderick and Harshfield, (4) using a different technique, report serum calcium determinations in thirty-three cases of acidosis of pregnant ewes, with serum phosphorus determinations in fifteen of the cases. In sixteen of these cases, or 48 per cent, the serum calcium was subnormal. Altho their technique produces a distinctly higher reading than does that used by the present writers, even with distinctly higher calcium readings, the Ca/P ratio is less than normal in all but two cases. Sampson, Gonzaga and Hayden (5) report serum calcium determinations in two cases of acidosis of pregnant ewes and in both, the serum calcium was subnormal. Greig, (12) without describing his technique, reports serum calcium determinations in six cases of acidosis of pregnant ewes, with maximal, minimal and average quantities distinctly less than the average quantity he found in normal sheep.

BLOOD TESTS OF HEALTHY PREGNANT EWES

For seventeen years the Kentucky Experiment Station has maintained a flock of sheep. During this time but one case of

TABLE 1. Blood Tests of Normal Pregnant Ewes at Term.
(Expressed as mg per 100cc of blood serum.)

Ewe	Ca	P	Ca/P	Sugar	Alkalinity*
1	8.9	3.9	2.2	———	———
2	9.2	4.5	2.0	———	———
3	9.3	3.7	2.5	———	———
4	9.4	7.0	1.3	53	1.00
5	9.7	3.0	3.2	———	———
6	9.9	4.7	2.1	88	———
7	9.9	3.2	3.1	———	———
8	9.9	6.0	1.6	68	0.60
9	10.0	4.0	2.5	71	0.95
10	10.2	5.0	2.0	46	0.85
11	10.3	4.7	2.1	65	0.73
12	10.4	4.7	2.2	55	1.10
13	10.4	4.7	2.2	55	0.95
14	10.4	5.5	1.9	57	0.92
15	10.4	5.2	2.0	———	———
16	10.5	4.7	2.2	65	1.10
17	10.9	4.7	2.3	60	0.75
Average	10.0	4.7	2.1	62	0.89

* Alkalinity in cc of .01N HCl per 1cc of blood serum, using phenolphthalein as indicator.

acidosis of pregnant ewes has occurred in this flock. In Table 1 blood tests of seventeen pregnant ewes of this flock, at or near term, are tabulated.

BLOOD AND URINE TESTS OF EWES WITH ACIDOSIS

While during the past seven lambing seasons we have examined sixty-two cases of acidosis of pregnant ewes, it was not possible to obtain both blood and urine from each case. However, during this period chemical examinations of both blood and urine were made in thirty-three cases. Tables 2 and 3 show the results of these examinations.

TABLE 2. Blood Tests of Ewes with Acidosis.
(Expressed as mg per 100cc blood serum.)

Ewe	Ca	P	Ca/P	Sugar	Alkalinity*
A 4906	3.3	4.8	0.7	-------	-------
A 4911	3.7	2.7	1.3	-------	-------
17375	4.6	4.7	0.9	-------	-------
Moore	4.7	6.0	0.8	-------	0.0
A 4910	4.8	5.0	0.9	-------	-------
17728	5.7	5.3	1.0	64	-------
A 4902	6.4	7.0	0.9	200	-------
19086	7.0	5.0	1.4	68	0.45
Ward 1	7.5	6.0	1.2	73	-------
17723	7.5	10.0	0.7	68	-------
L 3	7.6	5.2	1.4	92	-------
L 2	7.8	15.0	0.5	52	-------
18354	8.2	4.3	1.9	84	-------
18359	8.4	8.3	1.0	141	-------
19066	8.5	11.0	0.7	187	0.15
Ward 4	8.5	10.0	0.8	60	0.26
16	8.6	3.4	2.1	-------	-------
16715	8.6	7.5	1.1	-------	-------
18376	8.8	8.0	1.1	283	-------
17704	8.8	8.3	1.0	83	-------
14846	8.9	5.4	1.6	-------	-------
18369	8.9	10.0	0.8	68	-------
18319	9.1	6.0	1.5	60	-------
Wilson	9.1	9.0	1.0	88	0.53
18377	9.4	12.5	0.7	81	-------
19041	9.4	6.5	1.3	125	-------
Speed	9.5	11.0	0.9	250	-------
Hancock	9.5	12.5	0.7	79	0.0
17700	9.8	6.2	1.6	50	-------
17703	9.9	3.8	2.6	88	Recovered
17701	10.0	11.0	0.9	78	-------
18358	11.0	7.0	1.5	154	-------
19060	11.3	10.0	1.1	107	0.40
Average	8.0	7.5	1.1	107	0.26

* Alkalinity in cc of .01N HCl per 1cc of blood serum, using phenolphthalein as indicator.

TABLE 3. Urinalyses of Ewes with Acidosis.

Ewe	pH	Albumin	Ammonia Percent	Acetone Percent	Specific Gravity	Inorganic S mg per 100cc
A 4906	7.8	++	0.08	———	1045	69.8
A 4911	5.4	++	———	———	1015	54.8
17375	6.6	++	0.15	0	1045	625.0
Moore	8.0	++	0.01	Present	1030	59.5
A 4910	7.8	+	———	———	1029	101.2
17728	5.2	+	0.10	———	1020	4.3
A 4902	6.8	+	0.10	———	1008	20.0
19086	5.6	+	0.15	1.25	———	———
Ward 1	6.0	++	0.02	1.00	1025	61.4
17723	6.4	+	0.05	———	1014	29.5
L 3	5.4	+	0.02	———	———	4.7
L 2	5.2	+	0.05	———	1015	68.0
18354	5.2	+	0.10	———	1028	9.0
18359	5.2	++	0.05	———	———	15.8
19066	6.4	+	0.005	0	1015	29.8
Ward 4	6.8	++	0.07	1.00	1028	56.8
16	6.4	+	0.08	1.25	1037	———
16715	6.2	++	0.13	1.25	1020	15.9
18376	6.0	+	0.05	———	———	———
17704	6.2	++	0.10	———	1025	27.2
14846	5.2	Trace	0.02	2.00	1037	———
18369	6.0	++	0.10	———	1035	23.7
18319	6.0	+	0.10	———	1034	87.7
Wilson	6.4	+	0.20	1.00	1035	83.8
18377	5.0	++	0.10	———	1015	0
19041	5.6	+	0.10	1.00	1020	64.2
Speed	5.8	++	0.02	0	1025	81.9
Hancock	5.2	++	0.05	Present	1018	5.4
17700	6.4	+	0.10	———	1038	82.2
17703*	6.6	Trace	0.15	———	1019	27.4
17701	7.0	++	0.05	———	1022	38.7
18358	5.2	+	0.05	———	———	23.5
19060	6.0	++	0.01	Present	1016	35.9
Average	6.1	+	0.07	Present	1025	62.3
Normal	7.8	0	0.01	0	1020	28.0

* Recovered.

ADDITIONAL BLOOD AND URINE TESTS OF EWES WITH ACIDOSIS

In addition to those field cases of acidosis of pregnant ewes in which complete blood and urine analyses were made, there were a number in which the analyses were not complete. Tables 4 and 5 show the results of these analyses.

Acidosis is one of the important pathologic conditions which may occur in the ewe during the last two months of pregnancy. An ideal method for demonstrating acidosis would require the determination of the CO_2 of the alveolar air and of the blood, the titratable alkalinity of protein-free filtrate from the blood

TABLE 4. Blood Tests of Ewes with Acidosis.
(Expressed as mg per 100cc blood serum.)

Ewe	Ca	P	Ca/P
21	4.2	4.4	0.9
27	4.7	6.6	0.7
25	5.0	6.6	0.7
Pontius	5.9	10.0	0.6
15	6.2	4.5	1.3
17	6.6	4.8	1.3
19	6.6	4.5	1.4
17015	6.8	8.6	0.8
16720	6.8	7.5	0.9
26	7.2	10.3	0.7
20	7.2	4.4	1.2
Allen 1	7.5	12.5	0.6
18	7.5	6.5	1.1
23	7.6	5.0	1.5
13026	7.7	4.8	1.3
13366	7.9	6.7	1.2
29	7.9	5.3	1.5
24	8.0	7.5	1.0
28	8.0	5.1	1.5
13257	8.1	9.0	0.9
13367	8.5	7.9	1.0
Weil 1	8.9	9.5	0.9
13292	8.9	7.5	1.2
14816	9.3	8.6	1.0
13527	9.4	5.3	1.7
16468	10.0	10.0	1.0
14859	10.1	12.0	0.8
17702	11.0	9.2	1.2
Average	7.6	7.3	1.0

TABLE 5. Urinalyses of Ewes with Acidosis.

Ewe	pH	Albumin	Ammonia Percent	Acetone Percent
Ward 2	5.8	+	0.10	1.0
Ward 3	7.0	+	0.15	1.0
6988	7.6	+	0.15	1.0
6995	5.2	+	0.10	1.0
6997	5.0	+	0.16	1.0
7008	5.6	+	0.10	1.0
1	5.4	+	0.15	1.0
2	5.4	+	0.15	1.0
3	5.2	Trace	0.11	1.0
4	5.6	Trace	0.18	1.5
5	5.0	Trace	0.15	1.0
7	5.2	Trace	0.09	1.0
11	5.0	+	0.15	1.0
13257	——	——	0.17	1.0
13292	——	——	0.10	1.0
14816	——	——	0.04	1.0
Average	5.6	——	0.13	1.0

serum, the tolerance of the body to sodium bicarbonate, the presence of acetone bodies in the urine, and an increased output of ammonia in the urine; yet such an ideal method is not applicable in cases of acidosis of pregnant ewes. However, it is possible to determine the titratable alkalinity of the blood serum, the presence of acetone, and increased ammonia in the urine.

During metabolism the oxidation of carbon, phosphorus and sulfur compounds results in the constant production of acid radicles which are prevented from existing as free acid in the blood or other tissues chiefly by the processes of oxidation, excretion and neutralization. The CO_2 from the oxidation of carbon compounds is eliminated, as such, by the lungs and, in combination as urea, by the kidneys. The small amount of acid arising from oxidation of phosphorus and sulfur compounds is neutralized by the fixed bases of the body. The kidneys aid in regulating the reaction of the blood by excreting acid or alkaline carbonates and phosphates, as required. In the course of nitrogenous metabolism ammonia occurs and it is generally accepted that ammonia appears in the urine normally only to the extent required for the neutralization of acid. In most pathologic conditions elimination of CO_2 is readily accomplished if fixed bases are available for the transportation of CO_2 to the lungs. In elimination by the kidney the margin of safety is small. The reserve of fixed alkali which can be drawn upon without disturbing the body metabolism is not large and, under certain conditions, the process of neutralization by ammonia is used. Sellards (9) states: "The cardinal feature of acidosis is, that there is neither an increased production nor an accumulation of acid in the body, but the reserve supply of alkali-yielding substances is depleted in preventing the appearance of free acid in the blood. It is now established that fatal acidosis may occur with a normal or subnormal output of ammonia due to various types of kidney disease." Acidosis may occur during a disturbance of carbohydrate metabolism, during the course of nephritis, in the presence of advanced lesions of the liver, in the so-called food intoxications of children and during the last two

months of pregnancy. Tables 2 and 3 illustrate what one would expect to find. In the blood, diminished calcium, increased phosphorus, diminished Ca/P ratio, diminished alkalinity and occasionally increased sugar. In the urine, albumin always, increased ammonia, acetone almost always and, with few exceptions, acidity.

During the past few years, calcium metabolism has been studied by a number of investigators and Cantarow, who has recently published a critical study of these investigations, (13) states that the recognition of certain disease conditions in their incipiency, is a valuable contribution to the newer medicine. Among these are conditions dependent on nutritional disorders, those due to variations in endocrine function, those manifested by alterations of the acid-base equilibrium, and by disturbances of water balance. The importance of the inorganic elements, particularly calcium, sodium and potassium, in their relation to cellular metabolism and permeability seems evident.

Stewart and Percival (14) state that alterations in serum calcium during pregnancy are: "Doubtless due to the drain on the maternal tissues to meet the growing demand of the fetus, a demand which increases, according to Bosworth, Bowditch and Giblin, from 6 mg per day during the first four months of gestation to over 600 mg per day at term, and may average, according to Givens and Maey, 100 mg per day over the whole period. Corresponding to this rapidly increasing fetal requirement, the calcium in the maternal serum tends to fall." Milk fever in cattle represents a fundamental disturbance of calcium metabolism associated with lactation, and Fish (15) demonstrated, in eighteen cases, diminished serum calcium and serum phosphorus. Becker, Neal and Shealy (16) demonstrated that dairy cows dependent upon grass forage and silages grown on acid sandy soils containing relatively small amounts of calcium withdrew, during lactation, mineral matter from the bones to such an extent that the bones were weakened and easily broken. A 2-percent addition of bone meal to the concentrates, and a daily allowance of five pounds of alfalfa hay resulted in a material in-

crease in milk production, and minerals were stored to the point that the shaft bones were above average strength.

Investigators have shown that there is retention of calcium and phosphorus by the maternal organism during pregnancy, particularly during the later months of pregnancy, and this retention is associated with a tendency toward a diminution in the concentration of serum calcium. Some investigators believe that these alterations in mineral metabolism bear an important relation to the development of osteomalacia, tetany and eclampsia during pregnancy and lactation. On the basis of these and similar observations it would appear that calcium salts should be added to the dietary in the later months of pregnancy and especially during lactation. The introduction of calcium therapy into the management of the several clinical conditions associated with acute lesions in the liver constitutes a significant advance. In any case, upon any diet (during pregnancy and lactation), it is essential that calcium be present in amounts considerably above that required for maintenance of calcium equilibrium, in order to allow for the variable degree of absorption which is ordinarily uncontrolled. It has been found that the diets of higher calcium content, which permit more rapid calcification in the growing body, not only result in somewhat earlier maturity but also, in a greater measure, extend the period which lies between the attainment of maturity and the appearance of senescence. These observations emphasize the fact that normal growth cannot always be assumed to indicate normal development, and illustrate the importance of maintaining a high calcium intake during the period of growth.

EXPERIMENTAL ACIDOSIS

During the past five years a small flock of from seven to thirteen ewes has been maintained for experimental purposes. Originally, in June, 1928, ten average ewes, sixteen to eighteen months of age, were purchased from the Stock Yards. During the first two seasons, beginning in August each year, the flock was divided into two groups of five ewes each. Group One was fed three pounds each, per day, of a mixture of three parts of

corn, three parts of wheat bran and one part of linseed oil meal, with a liberal allowance of alfalfa hay. The other group was fed one pound each, per day, of a mixture of equal parts of corn and oats and a liberal supply of corn stover or poor timothy hay. Both groups were liberally supplied with water. From time of purchase in June the ewes were confined in dry lots

Fig. 3. Experiment Flock of Seven Ewes and a Buck.

until the close of the first lambing season, spring of 1929. After lambing and until September 1, 1929, the ten ewes were allowed to run in a small paddock in which pasture grass was very limited. From September 1, 1929, until close of the lambing season, spring of 1930, the ewes were on dry feed but allowed to run in a small paddock for exercise; there was very little pasture grass at any time. During the first season the serum calcium, potassium and phosphorus were determined in the blood of each ewe once each month until after lambing. During the second season similar determinations were made in June, November, December, and the following January. In the first season nine ewes produced eleven lambs, including two pairs of twins, and one failed to produce. In the second season all ten ewes produced lambs, including two pairs of twins, making a total of twelve lambs. Acidosis did not occur during the first two seasons.

Tables 6 and 7 show averages of the monthly blood tests of these ewes for the first and second seasons.

At the beginning of the third season, August, 1930, the experimental flock consisted of thirteen ewes; the ten original ewes and three ewe lambs produced the first season. All were placed together and fed the same. The feed consisted of one gallon of shelled corn per day for the thirteen ewes, a liberal supply of timothy hay, and water every other day. All the ewes were bred and proved pregnant. Two ewes that were on poor feed during the first two seasons died of acidosis: ewe A 4910

TABLE 6. Average Blood Tests of Five Ewes that Received Good Feed.
(Expressed as mg per 100cc of blood serum.)

Date	Ca	P	Ca/P	K
6/8/28	9.3	7.7	1.2	33.1
7/18/28	10.0	7.3	1.4	26.9
8/15/28	9.1	6.6	1.4	26.1
9/17/28	8.9	8.1	1.1	25.9
10/20/28	9.0	9.2	0.9	26.7
11/19/28	10.2	5.7	1.8	23.3
12/17/28	10.2	6.4	1.6	23.8
1/14/29	10.7	4.9	2.0	26.4
6/4/29	11.7	8.3	1.4	27.1
11/27/29	13.0	4.9	2.8	19.3
12/18/29	11.2	5.3	2.1	32.4
1/31/30	9.6	5.6	1.7	29.7
Average	10.2	6.7	1.6	26.7

TABLE 7. Average Blood Tests of Five Ewes that Received Poor Feed.
(Expressed as mg per 100cc of blood serum.)

Date	Ca	P	Ca/P	K
6/8/28	8.9	6.5	1.3	26.9
7/18/28	9.3	6.7	1.4	28.0
8/15/28	10.0	6.3	1.6	26.6
9/17/28	8.6	8.9	0.9	23.2
10/20/28	8.7	6.2	1.4	23.2
11/19/28	8.3	6.8	1.2	28.0
12/17/28	7.7	7.4	1.0	21.7
1/14/29	7.3	7.9	0.9	22.0
6/4/29	11.9	8.1	1.4	22.7
11/27/29	11.4	5.3	2.1	15.8
12/18/29	10.1	5.2	1.9	32.2
1/31/30	8.7	6.2	1.4	25.6
Average	9.2	6.8	1.4	24.6

died January 12, 1931, and ewe A 4911 died January 13, 1931. (See Table 9.) Both ewes produced twin lambs the first season, 1929, one lamb each the second season, 1930, and were carrying twins at the time of death. The remaining 11 ewes produced 16 lambs; 5 producing twins, and 6 one lamb each. Of the latter, two were dead at birth. Table 8 shows average blood tests for the eleven ewes that did not develop acidosis in the third season.

TABLE 8. Average Blood Tests of Eleven Ewes that Did Not Develop Acidosis.
(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
1/5/31	9.5	6.4	1.3	21.8
1/14/31	7.8	5.6	1.3	29.2
Average	8.6	6.0	1.3	25.5

Table 9 shows the average blood tests of the two ewes that developed acidosis during the third season.

TABLE 9. Average Blood Tests of Two Ewes that Developed Acidosis.
(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
1/5/31	8.7	7.2	1.2	19.4
1/12/31	4.8	5.0	1.0	26.4
1/13/31	3.7	2.7	1.4	25.5
Average	5.7	5.0	1.2	23.8

Eleven ewes were in the experimental flock at the beginning of the fourth season, August, 1931. During the season the flock was fed and managed the same as in the third season except that, beginning December 21, 1931, and continuing until January 12, 1932, ewes A 4905 and A 4908 were fed fresh carrots, ¼ lb. each, twice daily, in addition to the regular feed. The carrots were increased to ½ lb. each, twice daily, January 12, 1932, and were discontinued as soon as the ewes lambed. These two ewes were selected for feeding carrots because they showed the lowest

serum Ca and the lowest Ca/P ratio of all the ewes in the experimental flock. Ewes A 4905 and A 4908 gave birth to one lamb each on January 21, and January 25, 1932, respectively. Table 10 shows improvement in the blood tests of these ewes after the carrots were given.

TABLE 10. Average Blood Tests of Two Ewes Fed Carrots, Beginning December 2, 1931.

(Expressed as mg per 100cc of blood serum.)

Date	Ca	P	Ca/P
12/8/31	7.9	5.3	1.5
1/4/32	9.2	4.2	2.2
1/26/32	8.7	5.5	1.6
Average	8.6	5.0	1.7

Of the eleven ewes, all of which were bred, ten became pregnant. Two died of acidosis, ewe A 4906 on December 7, 1931, and ewe L2 on January 12, 1932. See table 13. Ewe A 4906 that was on good feed the first two seasons produced one lamb the first season, 1929, one lamb the second season, 1930, twin lambs the third season, 1931, and was carrying twins at the time of death. Ewe L2, added to the flock at the beginning of the third season, produced one dead lamb that season and was carrying twins at the time of death. The remaining eight pregnant ewes produced ten lambs, two produced twins, and 6, one lamb each. Ewe A 4902 produced one lamb January 26, 1932,

TABLE 11. Blood Tests of Ewe A 4902 During the Fourth Season.

(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
12/8/31	9.5	5.5	1.7	11.9
1/26/32	7.2	8.9	0.8	8.7
2/16/32	9.2	6.2	1.5	17.4
3/18/32	6.4	7.0	0.9	20.5
Average	8.1	6.9	1.2	14.6

Ewe A 4904 died of heat stroke July 16, 1932.

but did not do well following lambing and on March 18, 1932, died of pneumonia. The blood tests suggest acidosis, also.

Table 12 shows averages of blood tests for six ewes that did not develop acidosis in the fourth season. The two ewes that were fed carrots and the ewe that died of pneumonia are not included in table 12.

TABLE 12. Average Blood Tests of Six Ewes that Did Not Develop Acidosis.
(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
12/8/31	9.5	5.7	1.6	18.2
1/4/32	8.2	4.4	1.8	20.3
1/16/32	7.9	5.5	1.4	19.4
1/26/32	9.3	5.9	1.5	10.8
Average	8.7	5.4	1.6	17.2

Table 13 shows the average blood tests of the two ewes that developed acidosis in the fourth season.

TABLE 13. Average Blood Tests of Two Ewes that Developed Acidosis.
(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
12/4/31	3.3	4.8	0.7	6.8
12/8/31	9.3	6.9	1.3	13.7
1/11/31	7.8	15.0	0.5	15.6
Average	6.8	8.9	0.8	12.0

At the beginning of the fifth season, August, 1932, the experimental flock consisted of seven ewes. Four of these were placed on good pasture and were given, also, all the mangels and timothy hay they would eat. Three were kept on dry feed which consisted of all the timothy hay they would eat and one quart of shelled corn per day. The seven ewes were bred, but only six became pregnant. One ewe, L3, that was on dry feed died of acidosis. She was carrying twin lambs. See table 16. The remaining five pregnant ewes produced ten lambs, three of

them produced twin lambs, one a single lamb, and one, triplets, two of which were dead at birth. The third died the second day.

TABLE 14. Average Blood Tests of Four Ewes on Pasture, Timothy Hay and Mangels, that Did Not Develop Acidosis.
(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
8/9/32	10.7	6.3	1.6	28.3
9/26/32	10.1	4.8	2.1	16.1
10/24/32	10.1	7.3	1.3	24.6
11/28/32	10.9	5.8	1.8	14.0
12/27/32	10.6	5.0	2.1	6.5
1/30/33	9.6	4.4	2.1	28.5
Average	10.3	5.6	1.8	19.7

TABLE 15. Average Blood Tests of Two Ewes on Timothy Hay and Corn, that Did Not Develop Acidosis.
(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
8/19/32	10.8	6.5	1.6	28.2
9/26/32	9.5	4.6	2.0	18.7
10/24/32	9.3	7.2	1.2	17.7
11/28/32	10.2	5.2	1.9	11.2
12/27/32	9.6	5.0	1.9	6.8
1/30/33	9.2	5.0	1.8	29.1
Average	9.8	5.6	1.7	18.6

TABLE 16. Blood Tests of Ewe L3 that Developed Acidosis.
(Expressed as mg per 100cc blood serum.)

Date	Ca	P	Ca/P	K
8/9/32	10.6	5.5	1.9	29.1
9/26/32	9.3	5.2	1.8	18.5
10/24/32	9.6	7.5	1.3	18.4
11/28/32	11.2	5.2	2.1	11.9
12/27/32	10.0	6.2	1.6	11.4
1/11/33	7.6	5.2	1.4	20.6
Average	9.7	5.8	1.7	18.3

METHODS OF ANALYSES

Blood was obtained by inserting a sixteen-gage hypodermic needle into the jugular vein of the ewe and collecting 25cc of

blood in a chemically clean, dry test tube, 6 x ¾ inches. After clotting, the blood was separated from the sides of the tube with a clean, dry, slender glass rod, the tube securely corked, and placed in an ice-box at 11°C over night, during which time a sufficient quantity of clear serum separated from the clot.

For calcium determinations the Kramer and Tisdall method (18) was used; for potassium, the Kramer nad Tisdall method (19); for inorganic phosphorus, the Tisdall method (20), and for sugar, the Benedict and Osterberg method (21). The titratable alkalinity was determined by a modification of Sellard's method (22). To 1cc of serum in a 15cc centrifuge tube is added 2cc of 95% alcohol, the tube thoroly shaken, the precipitated protein thrown down in the centrifuge, the supernatant liquid decanted into an evaporating dish, three drops of a 0.5 percent phenolphthalein solution added, the liquid evaporated to dryness on the water bath and .01*N* HCl added, drop by drop, until all color is discharged, and the quantity of HCl required noted. For inorganic sulfur of the urine, 1cc of concentrated HCl is added to 10cc of urine in a clean, dry centrifuge tube and, after effervescence has subsided, the tube corked and allowed to stand over night. The preparation is centrifuged, the urine decanted into a clean, tared, graduated centrifuge tube, the quantity noted and 1cc of a 5 percent barium chloride solution added and, after standing one hour, the preparation is centrifuged and the urine decanted, 5cc distilled water added, the preparation again centrifuged, the water decanted and this washing with distilled water repeated once. The tube and precipitate are dried to constant weight. Weight of barium sulfate multiplied by 13.7 and divided by the number of cc of urine used gives the quantity of sulfur in milligrams per 100cc.

For urinary ammonia 1cc of fresh urine is diluted with 9cc of fresh tap water, and to 1cc of this dilution in a 6 x ¾ inch test-tube, 9cc of fresh tap water, five drops of .01*N* NaOH and 2cc of Nessler's solution are added, the tube corked and allowed to stand one hour. Urine containing a normal quantity of ammonia, about 0.01%, treated in this manner shows a small quantity of a light yellow precipitate. As the quantity of am-

monia in the urine increases the precipitate also increases in quantity and color making it possible to determine the quantity of ammonia present to a degree suitable for clinical purposes. Table 17 shows duplicate determinations by this method.

TABLE 17. Duplicate Ammonia Determinations.

Normal Urine	Added Ammonia	Determination
	Percent	Percent
245	0	0.01
245	0	0.01
245[1]	0.01	0.02
245[1]	0.01	0.02
245[2]	0.10	0.14
245[2]	0.10	0.14
242	0	0.03
242	0	0.04
242[1]	0.01	0.03
242[1]	0.01	0.03
242[2]	0.10	0.15
242[2]	0.10	0.15

TREATMENT

In the treatment of pregnant ewes which had symptoms of acidosis many different medicinal agents were tried without apparent beneficial results. Large doses of Epsom salts, administered as a drench or thru a stomach tube; enemas; intravenous injections of Epsom salts, calcium lactate, sodium bicarbonate, calcium chloride, calcium gluconate, insulin, paroidin and glucose were of no value. Dilatation of the os uteri and udder inflation were equally valueless. Chloral hydrate, ox bile and common salt, given by the mouth, were of no value. Early in our work with acidosis of pregnant ewes a number of cases were treated with botulinus antitoxin and the various preparations used for immunizing against hemorrhagic septicemia, without beneficial results. Two pregnant ewes with acidosis, which showed an inclination to eat, were fed bran, carrots, cane sugar and calcium gluconate and one of them recovered. This was the only case to recover under treatment. Sampson, Gonzaga and Hayden (5) suggest the intravenous administration of 30 gm. of glucose twice daily, if the case is seen soon after the onset, but they agree with us, (11) and with Roderick and Harshfield (4) that advanced cases are hopeless.

SUMMARY

In acidosis of pregnant ewes, with few exceptions, the urine is acid, contains albumin, acetone and increased ammonia. The blood calcium is lowered and the blood phosphorus increased so that the ratio Ca/P is always less than normal. There were but two exceptions to this in our sixty-one cases, and one of these recovered and was the only case that did recover. The condition is clearly an acidosis resulting from improper nourishment and care of ewes, and increased demand on the maternal calcium during the last two months of pregnancy. Advanced cases of acidosis of pregnant ewes appear hopeless, but the condition can be prevented by proper nourishment and care of ewes during pregnancy.

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